CHEMICAL UNDER CONSIDERATION FOR POSSIBLE LISTING VIA THE AUTHORITATIVE BODIES MECHANISM: IMAZALIL

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Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Imazalil may meet the criteria for listing as known to the State to cause reproductive toxicity and cancer under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Health and Safety Code Section 25249.5 et seq.), more commonly known as Proposition 65, via the authoritative bodies mechanism. The regulatory requirements for listing by the authoritative bodies mechanism are set forth in Title 22, California Code of Regulations, section 12306[1]. The regulations include the criteria for evaluating the documentation and scientific findings by the authoritative body that the Office of Environmental Health Hazard Assessment (OEHHA) uses to determine whether listing under Proposition 65 is required.

The U.S. Environmental Protection Agency (U.S. EPA) is one of five institutions that have been identified as authoritative bodies for identification of chemicals as causing reproductive toxicity for the purposes of Proposition 65 (Section 12306[l][4]). U.S. EPA has identified imazalil as causing reproductive toxicity. U.S. EPA has also been identified as an authoritative body for identification of chemicals as causing cancer for the purposes of Proposition 65 (Section 12306[m][4]), and has also identified imazalil as causing cancer. OEHHA has found that this chemical appear to be "formally identified" by U.S. EPA as causing these toxicities as required by Section 12306[d]. Imazalil is the subject of documents published by the authoritative body that identify the chemical as causing reproductive toxicity and/or cancer and indicate that the identification is a final action (U.S. EPA 1999a, 1999b, 2002, 2003, 2005). These documents specifically and accurately identify the chemical and the documents meet one or more of the criteria required by Section 12306[d][2]).

OEHHA also finds that the scientific criteria in regulation appear to have been satisfied for "as causing reproductive toxicity" (Section 12306[g]) and "as causing cancer" (Section 12306[e]) for imazalil. In making this evaluation, OEHHA relied upon the discussion of data by the authoritative body in making its finding that the specified chemical causes these toxicities. A brief discussion of the relevant toxicity studies providing evidence for the findings is presented below. Some of the discussion is taken verbatim or paraphrased from U.S. EPA source documents, which are part of the administrative record for these chemicals (U.S. EPA 1999a, 1999b, 2002, 2003). The statements in bold reflect data and conclusions that appear to satisfy the criteria for the sufficiency of evidence for reproductive toxicity (Section 12306[g]) or carcinogenicity

[1] All further references are to Title 22 of the California Code of Regulations unless otherwise indicated.

(Section 12306[e]). The full citations for the authoritative body documents are given later in this document.

Chemical under Consideration for Possible Listing under Proposition 65

Chemical	CAS No.	Toxicological Endpoints	Chemical Use	Reference
Imazalil	35554-44-0	cancer developmental toxicity	Systemic fungicide used post-harvest on bananas, citrus; pre-planting to treat barley and wheat seed; and in nonfood use for chicken hatchery treatments.	U.S. EPA (1999a, 1999b, 2002, 2003, 2005)

Imazalil (CAS No. 35554-44-0)

Endpoint for assessing risks of imazalil for acute dietary exposure in females 13-50 years of age and for short term inhalation occupational exposures - increased resorption and decreased fetuses in the rabbit developmental study (U.S. EPA 2003, page 7, U.S. EPA, 2002, page 42)

"Developmental toxicity was manifested by increased number of resorptions/litter, with subsequent decreases in numbers of live fetuses/litter at 10 and 20 mg/kg/day doses. The increased resorption and decreased number of fetuses per litter can occur after a single exposure (dose) and was also seen in studies with other species (mice). Therefore, this endpoint is considered to be appropriate for this (acute) risk assessment." (U.S. EPA, 1999a, page 4)

Increased incidence of combined malignant and benign tumors in male rats and mice.

Developmental Toxicity

The following study descriptions are adapted from U.S. EPA (2002)

Prenatal Developmental Toxicity Study – Rat (MRID 41026603)
In a developmental toxicity study, imazalil sulphate (99.9% purity) was administered to 24 Sprague-Dawley rats/dose by oral gavage in aqueous solutions at dose levels of 0, 40, 80 or 120 mg/kg/day from days 6 through 16 of gestation. Maternal toxicity was observed at all dose levels as evidenced by significantly decreased mean food consumption (9.8%, 17.1% and 18.7%, for the low, mid and high doses, respectively) during the dosing period. Developmental toxicity was manifested by a dose-related significant decrease in mean fetal weights in the mid (7.1%) and high (17.9%) dose groups compared to the controls. Other effects reported in the high dose group included a

significantly decreased mean litter size (11.2 vs. 13.9 for the control), a significantly decreased number of live fetuses/litter (11.1 vs. 13.8 for the control group), and a significantly increased number of resorbed fetuses/litter (3.7 vs. 0.4 for the control group). An increase in the number of fetuses (but not litters) with rudimentary extra ribs (6/247 vs. 0/333 for the control group) was noted in the high dose group. The study is classified Acceptable/Guideline.

Prenatal Developmental Toxicity Study – Rabbit (MRID 42593601)
In a developmental toxicity study, imazalil sulphate (98.2-100% purity) was administered to 15 female New Zealand Albino rabbits/dose by gavage at dose levels of 0, 5, 10 or 20 mg/kg/day from days 6 through 18 of gestation. Maternal toxicity was observed at 10 and 20 mg/kg/day as evidenced by significantly decreased body weight gain (54% and 95%, respectively, during gestation day [GD] 6-18), respiratory difficulty, increased resorptions and increased mortality (8/15 at 20 mg/kg/day). Food consumption was significantly decreased at the mid and high doses (18% and 23%, respectively during GD 6-18). Developmental toxicity was manifested by increased number of resorptions/litter, with subsequent decreases in numbers of live fetuses/litter at 10 and 20 mg/kg/day doses. No external visceral malformations or variations were reported. The study is classified Acceptable/Guideline.

Prenatal Developmental Toxicity Study – Mouse (MRID 44578201)
In a developmental toxicity study, imazalil sulphate (99.5% a.i.) was administered by gavage at 0, 10, 40, 80, or 120 mg/kg/day to pregnant mice (30 females/dose) on GD 6-16. Dams were sacrificed on GD 19. At 40 mg/kg, maternal toxicity was characterized by decreased body weight gains during treatment (↓13%,GD 6- 17) and reduced corrected body weight gains (↓23%, $p<0.05$). The decrease in body weight gain continued during the post-treatment period. At 80 mg/kg, maternal toxicity was manifested by the following: death of four dams during treatment (GDs 8-17); reduced mean body weights on GDs 17 and 19 (↓10-11%, $p<0.01$); reduced mean body weight gains (↓22%, days 6-17, $p<0.05$); decreased gravid uterine weights (↓18%, not statistically significant); reduced corrected body weight gains (↓20%, $p<0.05$); and reduced food consumption (↓10-15%, GDs 6-18, $p<0.05$ or 0.01). At 120 mg/kg, maternal toxicity was characterized by the following: death of ten dams during the administration period (days 8-17, $p<0.05$); clinical signs of toxicity such as, tremors (1 female), prostration and hypothermia (3 females each), convulsions (4 females), excitability (5 females), and piloerection (6 females, $p<0.05$); reduced mean body weights on days 17 and 19 (↓25-27%, $p<0.001$); decreased gravid uterine weights (↓48%, $p<0.001$); reduced mean body weight gains (↓42-59%, days 6-19, $p<0.05$ or 0.001); reduced corrected body weight gains (↓54%, $p<0.001$); and reduced food consumption (↓19-21%, days 6-18, $p<0.01$ or 0.001. The developmental toxicity observed at 120 mg/kg/day was manifested by increased number of resorptions ($p<0.05$), resorptions/dam ($p<0.05$), and postimplantation loss resulting in reduced litter size. These findings were noted in an earlier range finding study. The study is classified Acceptable/Guideline.

In another developmental toxicity study in mice (MRID 44567802), imazalil sulphate (98.2% a.i.) was administered by gavage at 0, 10, 40, 80, or 120 mg/kg/day to pregnant mice (30 females/dose) on GD 6-16. Dams were sacrificed on GD 19. The maternal toxicity lowest observed effect level (LOAEL) is 40 mg/kg/day, based on mortality, slightly decreased food consumption and uterus weight. The maternal toxicity no observed effect level (NOAEL) is 10 mg/kg/day. These effects were more prominent at the higher doses in addition to reduced body weight and body weight gain. There was slight developmental toxicity observed at 40 mg/kg/day manifested by increased resorptions, and reduced litter size. The developmental toxicity LOAEL is 40 mg/kg/day, based on increased resorptions. The developmental toxicity NOAEL is 10 mg/kg/day. The study is classified Acceptable/Guideline.

Reproduction and Fertility Effects - Rat

In a 2-generation reproduction study, imazalil (≥95.0%) was administered in the diet to a non-inbred strain of 24 Wistar rats per sex at approximately 0, 5, 20 or 80 mg/kg/day for 60 days prior to mating, through mating and lactation (females only). Only one litter per generation was produced. The parental toxicity LOAEL is 80 mg/kg/day based on body weight and body weight gain decreases and increased liver vacuolation in males. The parental toxicity NOAEL is 20 mg/kg/day. The reproductive toxicity LOAEL is 80 mg/kg/day and the NOAEL is 20 mg/kg/day based on the increased duration of gestation for the P0 and F1 females. Offspring toxicity consisted of a statistically significant decreased litter size at birth from the dams producing the F1 and F2 litters (54% and 51% of control values for F1 and F2, respectively) at the high dose. The number of dead pups at birth were also statistically ($p \leq 0.05$ to $p \leq 0.0001$) increased at the high dose in both generations. A nominal trend (statistical analysis was not conducted for trend) for decreased implantation sites in both generations was observed. The decreased number of implantation sites was statistically significant ($p \leq 0.05$) in the F2 females at the high dose.

In the 2-generation reproduction study discussed above, qualitative evidence of increased susceptibility of the pups to imazalil was observed. The parental systemic toxicity NOAEL/LOAEL was 20/80 mg/kg/day, respectively. The offspring toxicity NOAEL/LOAEL was also 20/80 mg/kg/day, respectively. However, the pup survival rate was adversely affected by the imazalil in the F2 generation from birth to postnatal date (PND) 4. The data in the study did not indicate when pup deaths occurred. In the absence of such data, it was assumed that pups were dying as a result of increased susceptibility to imazalil from the milk intake during lactation.

In a published study (Tanaka 1995), imazalil (99%) was administered to Crj:CD-1 mice (10/sex/group) at dietary doses of 0, 0.012, 0.024, or 0,048% (0, 120, 240, or 480 ppm equivalent to 19, 39 and 79 mg/kg/day in males and 26, 45 and 102 mg/kg/day in females during the preconception period) from 5 weeks of age of the F_0 generation to 9 weeks of age in the F_1 generation. In the F_0 generation, exploratory behavior (number of movements, movement time, total distance and number of turnings) at 8 weeks of age was significantly increased in males of the high dose group. Number of vertical activities was significantly increased in the mid dose group, and number of defecations was

increased in the low dose group. These effects did not appear to be dose related. Females were not affected. In the F_1 generation, with regard to neurobehavioral effects, surface righting reflex in all treated females, in the high dose male offspring group on PND 4 and in the mid dose group on PND 7 was significantly affected in a dose related manner**.** Swimming behavior of head angle in the high dose males and females at PND 4 was significantly affected in a dose related manner. Other neurobehavioral parameters were not affected. The number of turnings (exploratory behavior) in female offspring was significantly increased in the mid dose group, the other groups showed insignificant increase compared to controls. Other exploratory behavior parameters were not affected in males or females. There were some significant effects on multiple water T-maze performance in females, but not in males. By week 8 there were no effects on exploratory behavior in either sex. These results suggest that neurobehavioral effects can occur in mice exposed prenatally to imazalil in their diet.

Cancer

U.S. EPA (1999b, 2002, 2003, 2005) has concluded that imazalil is "likely to be a human carcinogen" based on combined malignant and benign liver tumors in male rats and mice and combined malignant and benign thyroid tumors in male rats. Imazalil was previously evaluated by U.S. EPA in 1994 and 1998. At the 1994 and 1998 reviews, U.S. EPA concluded that imazalil was carcinogenic to male mice. In 1994, U.S. EPA concluded that a two-year carcinogenicity study in Wistar rats was not adequate for assessing the carcinogenicity of imazalil and recommended a new two-year study be conducted at higher doses. At the time, imazalil was classified in Group C (possible human carcinogen). The final cancer assessment evaluation in 1999 that resulted in the "likely to be a human carcinogen" classification, included review of an additional set of rat bioassays (U.S. EPA, 1999b). Subsequent U.S. EPA documents on imazalil (U.S. EPA, 2002, 2003, 2005) utilize the Agency's classification of imazalil as "likely to be a human carcinogen." The rat and mouse bioassays considered by the U.S. EPA (1999b) final cancer assessment evaluation to provide the basis for imazalil's "likely to be a human carcinogen" classification are described briefly below.

Swiss albino mice (50/group/sex) were given imazalil via diet for 23 months. In male mice, a statistically significant increase in combined hepatocellular adenomas or carcinomas was observed. The incidences were reported as follows: adenomas: 5/50, 2/47, 14/50, 13/48 for control, low-, mid-, and high-dose animals, respectively; carcinomas: 5/50, 6/47, 6/50, and 11/48 and combined adenomas or carcinomas: 10/50, 8/47, 17/50, and 22/48 [$p<0.01$]. In female mice, the incidence of combined hepatocellular adenoma or carcinoma was increased but did not reach statistical significance (3/45, 5/48, 2/45, and 9/47). The incidence of hepatocellular carcinoma in female mice was 0/45, 2/48, 2/45, and 3/47 for control, low-, mid-, and high-dose animals, respectively. U.S. EPA (1999b) viewed the tumor response in female mice as supportive of that seen in males even though most of the observed tumors were adenomas. U.S. EPA also noted that the tumors in the mouse appeared at a dose that was "not particularly high," and that structural analogs of imazalil, namely etaconazole, uniconazole, cyproconazole and tebuconazole, induced tumors at the same site in mice.

Male and female Hannover SPF Wistar rats (50 animals/group/sex) were exposed to imazalil via diet for two years. Thyroid and liver tumors were observed in male rats. The combined incidence of thyroid follicular cell adenoma or carcinoma (4/48, 8/47, 6/50, 11/49, and 12/49 for control, low-, midlow-, midhigh- and high-dose groups, respectively) was significantly increased ($p<0.05$) in the two highest dose groups and was outside the historical control range for these tumors. The incidence of thyroid follicular cell carcinoma was 0/48, 0/47, 2/50, 2/49, and 2/49. The incidence of combined hepatocellular adenoma and carcinoma (4/48, 2/47, 3/50, 4/49 and 13/49) was also significantly ($p<0.05$) increased in male rats. No treatment-related tumors were observed in female rats.

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